

Results: Expression of DIO2 in articular cartilage was significantly affected by the rs225014 risk allele and joint type ($P = 3.6 \times 10^{-3}$ and $P = 4.2 \times 10^{-2}$, respectively). Furthermore, we observed a single CpG dinucleotide 2 kb upstream of the DIO2 promoter that displayed a significant positive correlation with expression of DIO2 ($P = 3.3 \times 10^{-3}$). In addition, this CpG dinucleotide appeared significantly hypermethylated in OA cartilage, compared to matched preserved cartilage ($P = 1.6 \times 10^{-4}$) (Figure 1). Subsequent stratification for rs225014 genotypes revealed an enhanced relation between methylation and expression in carriers of the rs225014 risk allele. Further stratification for joint type did not reveal any major differences. ChIP on multiple chondrocyte cell lines identified a CTCF binding site directly on the location of the identified CpG.

Conclusions: Compelling regulatory properties of a single CpG dinucleotide 2 kb upstream of the DIO2 promoter seem responsible for the observed up regulation of DIO2 expression in OA cartilage, independent of rs225014 genotypes. However, expression of DIO2 is additionally modulated by rs225014 genotypes. As a result expression of DIO2 in risk allele carriers is more sensitive to changes in methylation, indicating an additive effect, which in turn could be causal to OA susceptibility.

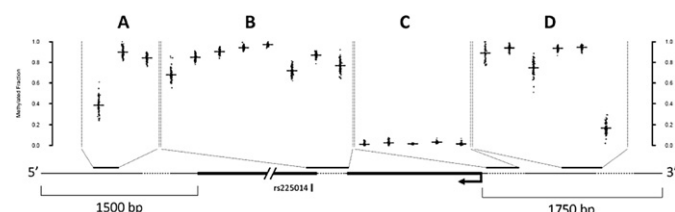


Figure 1. Overview of interrogated CpG dinucleotides in the DIO2 locus. The sixth CpG dinucleotide from region D associates significantly with DIO2 expression ($P = 0.0033$) and is differentially methylated between preserved and OA cartilage ($P = 1.6 \times 10^{-4}$).

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GENETIC RISK FACTORS FOR KNEE OA DISPLAY GOOD PREDICTIVE POWER IN A HIGH RISK GROUP OF OVERWEIGHT AND OBESE FEMALES

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Purpose: Over the last few years, several genetic markers have been identified to be robustly associated with knee osteoarthritis (OA). These genetic markers have been identified by gathering all cases and controls available. In general, small effect sizes of these risk factors were found, making OA genetic risk prediction difficult. However, it is known that the association of the genetic marker with the phenotype can be context specific. It could be that in persons with or without major environmental risk factors, different sets of genes are important. We therefore set out to test predictive value of DNA variants previously identified as being associated to knee OA, in a high risk group of overweight and obese, middle-aged women.

Methods:

Study Sample: This study was performed within the *Prevention of knee Osteoarthritis in Overweight Females (PROOF)* study. A total of 407 women met the inclusion criteria (BMI ≥ 27 , no clinical knee OA (ACR criteria), no contraindications to MRI, no rheumatic diseases), were invited for baseline measurements. Pre-specified primary outcome was incidence of knee OA, defined by incidence of either K&L ≥ 2 , joint space narrowing of ≥ 1.0 mm or incident clinical knee OA (ACR criteria). After 2.5 years of follow-up, forty-three women (11%) were lost to follow-up and for a further 72 women no DNA was available.

DNA-variants: We studied DNA variants that have been reported as being associated with knee OA at genome-wide significance level ($P < 5 \times 10^{-8}$) in women. These included variants in or near: GDF5 (rs143383), GNL3 (rs11177) and DUS4L (also referred to as the Chr 7 locus, rs4730250) and MCF2L (rs11842874). The assay for the MCF2L variant failed due to technical problems. We subsequently computed a genetic risk score for the 3 variants that were genotyped successfully, where the number of carried risk alleles were added per person. Association between the genetic markers, the genetic risk score and

incident knee OA was tested by multivariate logistic regression analysis, adjusting for age and BMI.

Results: In total 92 (31%) women developed OA during the 2.5 year follow-up time. When the risk for the separate DNA variants were tested, for all three variants we found increased risk for incident knee OA with the previously identified risk allele (OR(95% CI): GDF5: 1.30 (0.91-1.85); GNL3: 1.23(0.86-1.78); Chr7: 1.75(1.10-2.77)). All these risks were at least twice as large as compared to those previously reported in large meta-analysis. However, only the odds ratio for the chr 7 locus reached nominally significance ($p=0.018$). The genetic risk score, which was composed of the three SNPs under study, was significantly associated with incident knee OA ($p=0.007$). With each additional risk allele, the women had 38% more chance for incident knee OA. When we stratified the women into 3 groups according to their genetic risk score, we observed that women with 4 or 5 genetic risk alleles had almost 3.5 times higher risk for incident knee OA compared to women that had 0 or 1 risk allele (OR 3.45 (95%CI 1.41-8.47), $p=0.007$).

Conclusions: The previously identified genetic risk markers for knee OA, were significantly associated with incident knee OA in this high risk group of overweight and obese women. The effect sizes of the studies DNA variants were larger than those reported previously. These results indicate that genetic prediction of OA can be dependent on the presence of other strong clinical risk factors

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THE MTDNA HAPLOGROUPS INFLUENCE THE PROGRESSION OF OSTEOARTHRITIS (OA). DATA FROM THE OSTEOARTHRITIS INITIATIVE (OAI)

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Purpose: The Osteoarthritis Initiative (OAI) is a multicentre study focused on identifying and evaluating risk factors of knee osteoarthritis (OA). Previous studies carried out by our group showed the role of the mitochondrial DNA (mtDNA) haplogroups on the prevalence and progression of OA. The aim of this study is to replicate our previous findings about the influence of the mtDNA haplogroups on OA progression in the well characterized cohort of the OAI.

Methods: Data for these analyses are from the OAI public use data set(s), and assigned the mtDNA haplogroups in 891 Caucasian samples of the progression subcohort.

We analyzed the influence of mtDNA haplogroups on OA progression attending to KL grade, joint space narrow (JSN), presence of osteophytes and subchondral sclerosis in medial tibial compartment, between baseline and visit 6 (48 months). The progression criteria for KL grade consisted in an increase of at least one (KL or OARSI) grade to any visit and any knee; for JSN we considered progression if an increase in score by a grade above or equal to 0.5 of the OARSI scale to any visit and any knee occurred. Additionally we also analyzed the influence of these mitochondrial polymorphisms on quantitative measurements of cartilage morphology from magnetic resonance imaging (MRI) scans: mean cartilage thickness and volume of cartilage in medial tibia. Appropriate statistical analyses adjusting by gender, age and body mass index (BMI) at baseline were carried out using SPSS software (v.19).

Results: Carriers of the mtDNA haplogroup T are at lower risk for KL grade progression (27.1 % progressors) than carriers of the most common mtDNA haplogroup H (46.9% progressors) (OR=0.442; CI=0.233-0.835; $p=0.012$). As expected, carriers of the haplogroup T are also better progressors, attending to osteophytes (35.2% progressors) and subchondral sclerosis (30.2% progressors) in tibia medial compartment, than carriers of the haplogroup H (53.4% progressors and 45.8% progressors respectively) (OR=0.481; CI=0.258-0.897; $p = 0.021$ and OR=0.525; CI=0.275-1.004; $p = 0.05$ respectively). Regarding to JSN, carriers of the haplogroup T also showed a better progression (23.3% progressors) than carriers of the haplogroup H (36.9% progressors) (OR=0.510; CI=0.261-0.995; $p = 0.048$).

The analyses of quantitative measurements of cartilage morphology revealed that carriers of the haplogroup T showed a higher mean cartilage thickness in medial tibia (1.88 mm \pm 0.28) than carriers of the haplogroup H (1.74 mm \pm 0.27) (borderline $p = 0.077$), as well as higher